Research

Control of *Pseudomonas amygdali* pv. *loropetalii* on Metal, Wood, and *Loropetalum chinense* Stem Surfaces

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Abstract

Clorox Germicidal Bleach, Cuproxat, Green-Shield II, KleenGrow, Virkon S, and ZeroTol 2.0 were evaluated for their ability to inactivate *Pseudomonas amygdali* pv. *loropetalii* on stainless steel (SS), pressure-treated wood (PtW), and loropetalum stem sections. Clorox at 11% product and Virkon S at 1.0% product eliminated *P. amygdali* pv. *loropetalii* on SS surfaces and nearly eliminated it on PtW surfaces. Green-Shield II at 0.5% product and KleenGrow at 0.8% product nearly eliminated the bacterium on SS, while causing a significant reduction without elimination on PtW. Cuproxat and ZeroTol 2.0 were not effective against this

bacterium in these applications. Clorox and KleenGrow were evaluated further for their ability to kill *P. amygdali* pv. *loropetalii* on loropetalum stems, which was used as a preliminary surrogate for vegetative stem cuttings. Only Clorox eliminated bacteria from stem surfaces, but the bacterial inoculum level appeared to affect efficacy. Several disinfectants are commercially available that kill *P. amygdali* pv. *loropetalii* on production surfaces.

Keywords: chemical control, horticultural crops, ornamentals, disinfectant, bacteria, disinfectant, sanitation

Pseudomonas amygdali pv. *loropetalii* was first identified in 2013 as the pathogen that causes bacterial gall on loropetalum cultivars (*Loropetalum chinense* [R. Br.] Oliv.) (Conner et al. 2013; Harmon et al. 2018). Disease symptoms include galls with a mean diameter of 1.2 cm on small to moderate-size limbs at the lower- to mid-canopy levels, with a progression of foliar nutrient deficiency symptoms, leaf dehiscence, and stem dieback distal of the gall over several months. The disease has become a problem in commercial plant nurseries in the southeastern United States and already present in the landscape in the same region.

The pathology and control methods for Loropetalum bacterial gall will need to be extrapolated from related diseases that have been around for years, such as *P. syringae* diseases, particularly olive knot caused by *P. syringae* pv. *savastanoi* (= *P. savastanoi* pv. *savastanoi*) (Kennelly et al. 2007; Ramos et al. 2012). *P. syringae* pv. *savastanoi* invades plant tissue through natural and inflicted wounds, such as leaf fall, hail, pruning cuts, and so on (Hewitt 1938; Marchi et al. 2005, 2009). The bacterium can fill intercellular plant

spaces, causing cell collapse and proliferation that both contribute to gall formation (Marchi et al. 2009; Surico 1977). Marchi et al. (2009) also found *P. syringae* pv. *savastanoi* cells in xylem tissue not associated with a gall that demonstrated systemic movement. Secondary tumors may result partly from systemic movement (Penyalver et al. 2006). The risk of systemic movement resulting in the presence of bacterium in asymptomatic stems used for vegetative propagation is unknown. As galls develop, *P. syringae* pv. *savastanoi* cells egress or are extruded onto the stem's phylloplane during high humidity and periods of rainfall, at which point bacteria can be rain-flow and rain-splash dispersed to other infection sites within the tree and to neighboring trees (Horne et al. 1912; Quesada et al. 2010a). *P. syringae* pv. *savastanoi* also multiplies on the phylloplane of stems and leaves while in a saprophytic phase (Ercolani 1978; Quesada et al. 2007). The bacterium can survive over several seasons in tree galls (Wilson 1935). Thus, infected and infested host material is considered a primary source of *P. syringae* pv. *savastanoi*. Loropetalum, like olive, is primarily propagated from vegetative stem cuttings, probably with a similar risk to spread *P. amygdali* pv. *loropetalii* (Wilson and Magie 1964).

Control of bacterial diseases on fruit and ornamental crops is difficult (Kennelly et al. 2007). Currently, copper compounds are the only practical chemical class recommended for landscape and container-grown loropetalum, as well as for rooted cuttings (Hagan and Conner 2013). However, copper compounds can cause crop injury under certain weather conditions (McManus et al. 2002; Sundin and Bender 1993), and bacteria have moderate potential to develop resistance to copper (Quesada et al. 2010b). Because copper compounds can only reduce epiphytic bacterial populations from plant surfaces, it is recommended to discard container-grown loropetalum stock that shows symptoms (Hagan and Conner 2013).

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The primary control efforts are to eliminate inoculum sources, thus preventing dissemination of *P. amygdali* pv. *loropetalii* onto clean loropetalum stock. The most significant level of control would be production of pathogen-free plant material where additional control is unnecessary. This is more easily achieved when the pathogen is not present in or around the production site. In areas where the pathogen is present or could potentially be introduced, it is advisable to disinfect pruning tools and production surfaces, along with scheduled isolation of newly acquired plant material. Once the disease is present in production blocks of plants, then more drastic controls would be required, such as disinfecting vegetative stem cuttings. Nguyen et al. (2017) found a quaternary ammonium compound (QAC) containing alkyl diethylbenzyl-, didecyl dimethyl-, octyl decyl dimethyl-, and dioctyl dimethyl-ammonium chlorides was more effective at reducing *P. syringae* pv. *savastanoi* from surfaces of motorized pruning hedgers soiled with macerated olive tissue than sodium hypochlorite, polyhexamethylene biguanide hydrochloride, chlorhexidine acetate, and a QAC that contains *n*-alkyl dimethyl benzyl- and *n*-alkyl dimethyl ethylbenzyl-ammonium chlorides. Pscheidt and Bassinette (2013) found that five applications of various treatments, including a peroxygen (ZeroTol 2.0), a QAC (KleenGrow), and a copper compound (Camelot O), did not control disease on lilac shrubs inoculated with *P. syringae* pv. *syringe*. No other studies were found that involved disinfectant activity on *P. syringae* plant pathogens.

The goal of this study was to evaluate efficacy of commercially available disinfectants and a copper compound against *P. amygdali* pv. *loropetalii* on surfaces of stainless steel (SS), pressure-treated wood (PtW), and loropetalum stems. At this point, loropetalum stems were included only to determine basic product efficacy levels on multiple types of substrates. The goal was not to fully assess the practicality of disinfecting stem cuttings, because other factors could play a role in efficacy. For example, seasonal distribution and density patterns of *P. amygdali* pv. *loropetalii* populations on stems of container-grown loropetalum could influence efficacy. Potentially, a lower rate of disinfectant could provide good efficacy with less phytotoxicity risk when bacterial density levels were low, whereas higher bacterial densities could result in less favorable sanitation outcomes. Further investigation will be necessary to more comprehensively evaluate potential of treating stem cuttings.

Pathogen Recovery and Identification

Three isolates were recovered from *Loropetalum* shrubs purchased at a local retail outlet in Forest County, Mississippi. Excised stem galls were surface disinfested with 10% bleach for 1 min, the bark was removed with flamed scalpels, and the internal wood was cut into pieces in 0.5 ml of sterile reverse-osmosis water (RO water) to soak for 1 h. Gall extracts were loop-transferred onto King's B agar (KB) (King et al. 1954) and incubated at room temperature until colonies were visible. Pure cultures were obtained by passaging the isolates on KB and then storing them at -75°C in a

medium supplemented with 30% glycerol. The strains were designated AAC, AAD, and AAE and were characterized using the multilocus sequence typing (MLST) scheme of Hwang et al. (2005). Briefly, genomic DNA was extracted from overnight KB cultures with a DNeasy UltraClean Microbial Kit (Qiagen, Germantown, MD) and subjected to polymerase chain reaction (PCR) with DreamTaq DNA polymerase (Thermo Fisher Scientific, Waltham, MA) and primers targeting the housekeeping genes *gapA* (encoding glyceraldehyde-3-phosphate dehydrogenase), *gltA* (citrate synthase), *gyrB* (DNA gyrase), and *rpoD* (sigma factor 70 of RNA polymerase). The amplicons were purified with a GeneJet PCR Purification kit (Thermo Fisher Scientific) and sequenced at Eurofins MWG Operon (Huntsville, AL). The DNA sequences were trimmed and concatenated in Geneious version 10.2.3 (Biomatters, Auckland, New Zealand), and aligned with MUSCLE (Edgar 2004). Homologous sequences from other species of the *P. syringae* complex were obtained from the Plant-Associated Microbes Database (PAMDB) (Almeida et al. 2010). Bootstrapped phylogenies were inferred with Geneious Tree Builder using the neighbor-joining algorithm and DNA distances corrected by the Jukes-Cantor genetic distance model (Jukes and Cantor 1969). The resultant phylogenetic trees were edited in iTOL version 4 (Letunic and Bork 2019). Housekeeping gene sequences generated in this study were deposited in NCBI GenBank and assigned accession numbers (Table 1).

Analysis of housekeeping gene sequences revealed that the isolates from loropetalum galls belong to the *P. syringae* group. *P. syringae* was first described over 100 years ago (Van Hall 1902) and since then has become recognized as an extended complex of species and pathovars that encompasses most of the phytopathogenic members of the genus *Pseudomonas* (Bull et al. 2010; Parte 2018). Use of molecular techniques has led to a series of taxonomic revisions based on results of DNA-DNA hybridizations (Gardan et al. 1999), DNA fingerprinting (Weingart and Volksch 1997), phylogenetic analysis of housekeeping genes (Hwang et al. 2005), and whole-genome comparisons (Gomila et al. 2017). Among these approaches, MLST based on the sequences of three or four housekeeping genes emerged as a method of choice for the rapid identification of species and pathovars of the of *P. syringae* group (Berge et al. 2014; Bull et al. 2011). MLST typing and comparisons against the PAMDB (Almeida et al. 2010) revealed that, at the species level, the loropetalum isolates most closely clustered with *P. amygdali* (Fig. 1). The earlier DNA-DNA hybridization studies placed *P. amygdali* into genomospecies 2 and suggested that this species should be considered synonymous with *P. savastanoi*, *P. ficuserectae*, and *P. meliae* (Gardan et al. 1999). These findings were further confirmed by a recent phylogenomic study of Gomila et al. (2017), who analyzed 139 complete and draft genome sequences of the *P. syringae* group and separated them into over 60 pathovars representing 15 recognized and at least seven novel bacterial species. That study delineated *P. amygdali* into a distinct

TABLE 1
GenBank accession numbers assigned to four conserved housekeeping genes of the three *Pseudomonas amygdali* pv. *loropetalii* strains used in the multilocus phylogenetic analysis

Strain designation	Genes			
	<i>gltA</i>	<i>gyrB</i>	<i>gapA</i>	<i>rpoD</i>
AAC	MN477935	MN477938	MN477941	MN477944
AAD	MN477936	MN477939	MN477942	MN477945
AAE	MN477937	MN477940	MN477943	MN477946

genomic branch (IV), along with four synonymous nomenclatures: *P. savastanoi*, *P. ficuserectae*, *P. meliae*, and *P. tremiae*.

The MLST typing of our loropetalum isolates AAC, AAD, and AAE revealed their close relatedness to *P. amygdali* pathovars that cause galls or cankers in woody organs of various plants. Although the virulence traits that enable infection of woody organs are poorly understood, it is known that these pathogens affect several

economically important species of trees and shrubs, including stone fruits (pvs. *syringae* and *morsprunorum*) and olive (pv. *savastanoi*) (Berge et al. 2014; Gomila et al. 2017). This group also includes the causative agent of the bacterial gall of loropetalum, which was first isolated by Conner et al. (2013) and identified as *P. savastanoi*. Recently, this pathogen was studied in more detail and described as a new pathovar of *P. amygdali* by Harmon et al. (2018). The authors

FIGURE 1

Multilocus phylogeny of loropetalum isolates used in this study. The three *Pseudomonas amygdali* pv. *loropetalum* strains (AAC, AAD, and AAE) are shown in bold, whereas phylogenomic species defined by Gomila et al. (2017) are highlighted with colors. The neighbor-joining phylogeny was inferred from concatenated nucleotide sequences (2,010 characters) of housekeeping genes *gapA*, *gltA*, *gyrB*, and *rpoD*. Evolutionary distances were estimated using the Jukes–Cantor genetic distance model. The reproducibility of clades was assessed by bootstrapping, and bootstrap values greater than 50% are indicated by gray circles at the nodes (circle sizes are proportional to bootstrap values).

analyzed 34 stem gall isolates from Florida and Alabama that infected only loropetalum while not producing galls on olive, almond, or mandevilla. The subsequent profiling with the levan production, oxidase reaction, potato soft rot, arginine dihydrolase activity, and tobacco hypersensitivity tests (LOPAT) (Schaad et al. 2001), Biolog, fatty acid, and MLST revealed that these strains represent a new pathovar, *P. amygdali* pv. *loropetalii*. The concatenated housekeeping gene sequences of *loropetalum* isolates used in this study were 100% identical to their homologs from G13-208 (= DSMZ 105780P^T) (Fig. 1), a designated pathotype strain of *P. amygdali* pv. *loropetalii* (Harmon et al. 2018).

Disinfestant Efficacy on Stainless Steel

Six chemical treatments were evaluated for activity against *P. amygdali* pv. *loropetalii*: (i) sodium hypochlorite (8.3% active ingredient [a.i.]) (Clorox Germicidal Bleach, The Clorox Co., Oakland, CA) at rates of 5.6 and 11.1% product; (ii) hydrogen peroxide (27.1%) and peroxyacetic acid (2.0%) (ZeroTol 2.0, Biosafe Systems, East Hartford, CT) at rates of 1% (1:100 ratio) and 2% (1:50 ratio) product; (iii) *n*-alkyl (60% C₁₄, 30% C₁₆, 5% C₁₂, 5% C₁₈) dimethyl benzyl ammonium chloride (10% a.i.) and *n*-alkyl (68% C₁₂, 32% C₁₄) dimethyl ethylbenzyl ammonium chloride (10% a.i.) (Green-Shield II, Whitmire Micro-Gen Research Laboratories, BASF Corp., St. Louis, MO) at rates of 0.5% (1 tsp per 1 qt) and 1.0% product; (iv) didecyl dimethyl ammonium chloride (7.5%) (KleenGrow, Pace 49, Burnaby, BC, Canada) at rates of 0.8 (1 fl. oz. per 1 gal) and 1.6% product; (v) potassium peroxymonosulfate (21.41%) and sodium chloride (1.50%) (Virkon S, E.I. du Pont de Nemours and Co., Dupont Animal Health Solutions, Wilmington, DE) at rates of 0.5% (0.65 oz per 1 gal) and 1.0% product (1.3 oz per 1 gal); and (vi) basic copper sulfate (27.1%) (Cuproxat, Nufarm Limited, Morrisville, NC) at 0.5 and 1.0% product. All treatment rates were stated as percent product of 100-ml solution that is calculated by addition of a dry soluble product directly to the RO water (w/v) and by removal of an equal volume of RO water prior to adding liquid product (v/v). Additionally, two negative controls were applied: no treatment (NoSpray) and sterilized RO water (WaterSpray). The WaterSpray treatment was done to assess the dislodging potential of bacteria during the spray application process. Chemical tests were run at the Thad Cochran Southern Horticultural Laboratory, Poplarville, Mississippi.

A randomized complete block design was used with three and four replications per experiment 1 and 2, respectively. Each treatment was independently taken through the inoculation, treatment application, and bacterium recovery sequence with a complete replication set done per day. All steps except spraying were performed in a class II biological safety hood. A single *P. amygdali* pv. *loropetalii* isolate (AAE) was used in all studies. Three- to 5-day-old cultures grown on KB at 26°C were used to make bacterial suspensions. Cultures were passaged a total of three times after plating from frozen stock. Bacteria were suspended in Ringer's solution and adjusted to an optical density of 0.3 at 600 nm (OD₆₀₀). SS 2.5-cm washers, of 793 mm³ surface area, were inoculated by placing them in the bacterial suspension in a 50-ml centrifuge tube for 2 min on a rocker table pivoting at a 1 s tilt rhythm. An inoculated SS washer was set in a 7.6 by 12.7 cm aluminum tray to dry for 15 min and then transported in a Bel-Art polypropylene sterilizing tray with cover (15-249A) to a chemical fume hood. Disinfestants were applied with a single 500-ml Kontes TLC sprayer at 6 psi and held 30.5 cm from the tabletop. Spray was applied in three back-and-forth moderate-speed sweeps to thoroughly wet material surfaces, and then the SS washer was flipped with forceps and the other side

sprayed as described. Between each application, the sprayer was triple rinsed with sterile RO water and the final rinsate sprayed out for 30 s. The SS washer was air dried for 8 min in the biosafety hood, after which the pathogen was recovered by vortexing the washer in 10 ml of Ringer's solution for 1 min and enumerated by serial dilution plating on KB agar. Cultures were grown at 26°C for 3 days and colony forming units (CFUs) counted using a Reichert Darkfield Quebec Colony Counter (RE-3325).

Recovery of *P. amygdali* from treated SS washers was evaluated using two statistical procedures, because some treatments resulted in a mean recovery and variance of 0 CFU. For the first analysis, recovery of CFU data from each SS washer was categorized as No or Yes if zero or greater than zero, respectively. The binary data were evaluated using the PROC FREQ procedure (SAS 9.4). The binomial option was used in both the tables and output statement to specify exact Clopper-Pearson confidence limits for binomial proportions based on $P = 0.5$. Treatments were considered to be not significantly different if binomial probability distributions overlapped. A second analysis was performed on the numerical data if two or more replications had CFUs greater than zero. Data were log transformed to obtain a normal distribution. Transformed CFUs were modeled with experiment and treatment as independent variables and replication as a random variable using the analysis of variance procedure PROC MIXED. Fisher's least significant test option of the LSMEANS procedure was used to determine pairwise differences ($P = 0.05$).

Based on binomial probabilities, both rates of Clorox, Green-Shield II, and Virkon S and the lower rate of KleenGrow were significantly lower than the NoSpray and WaterSpray treatments (Fig. 2). Of these treatments, only both rates of Clorox and the label (higher) rate of Virkon S resulted in 0 CFU in all replications. However, six of seven replications resulted in 0 CFU for both rates of Green-Shield II and for one rate of Virkon S and KleenGrow. Although zero recovery is the preferred outcome, caution is advised in distinguishing between zero and very low recovery incidences, because a large number of replications is needed to confidently separate these differences. The wide spread of the binomial probability distributions in Table 2 reflects the use of only seven replications. Despite the width of the distributions, these treatments resulted in a large reduction in log CFU and are viable control treatments (Table 2).

FIGURE 2
Binomial probability distribution of recovery of *Pseudomonas amygdali* pv. *loropetalii* from stainless steel washers sprayed with a disinfectant, water, or nothing.

Absolute control is not guaranteed with disinfectants, so it can be worthwhile to follow precleaning, rate, and contact time recommendations on product labels. Nguyen et al. (2017) found that two QACs, Deccosan 315 at 2.0% product and Deccosan 321 at 0.9% product (each at 2,000 µg of a.i./ml of water), successfully and sometimes completely prevented new infections from hedging shears soiled with contaminated plant tissue, whereas sodium hypochlorite at 0.2% product (100 µg of a.i./ml of water) was not effective. Both Green-Shield II and Deccosan 315 are third-generation QACs with a similar mixture of active ingredients, whereas KleenGrow and Deccosan 321 are fourth- and fifth-generation QACs, respectively. In both this and the Nguyen et al. (2017) studies, the QACs showed similar control activity on metal substrates. The results with sodium hypochlorite in the

Nguyen et al. (2017) study could not be compared with this study because Nguyen et al. used Clorox at a much lower rate and on unclean surfaces.

The five treatments with consistent recovery of log CFU that were not discussed in the previous paragraph were further analyzed by an ANOVA (Table 2). Due to heterogeneous variances, the highly conservative Dunnett's T3 method was performed based on the studentized maximum modulus that implements multiple adjustments to protect the overall error rate. The independent model variables and their interactions were significant: experiment (Exp, $P = 0.0001$), treatments (Trt, $P < 0.0001$), and Exp $\times$ Trt ($P = 0.0018$). However, only the higher rate of Cuproxat, KleenGrow, and ZeroTol 2.0 in experiment 2 were different from the NoSpray and WaterSpray treatments (Table 2). Because differences occurred only in one of two experiments, the differences were not biologically dependable, and these treatments should not be considered as adequate disinfestation control. The NoSpray and WaterSpray treatments in both experiments were not different from each other.

Other studies have shown sodium hypochlorite effective at inactivating bacteria on metal surfaces to variable degrees (Lane et al. 1948; Nichols and Jodon 1972; Teviotdale et al. 1991). To our knowledge, no studies evaluating the activity of QACs and peroxygen disinfectants on bacteria on metal surfaces have been published. Henn (personal communication) obtained similar results with chlorine, QAC, and peroxygen disinfectants against *Xanthomonas arbuticola* pv. *pruni* on metal pruners; interestingly, they found significant inactivation from additional products as well.

Disinfectant Efficacy on Pressure-Treated Wood

The same six chemical treatments and procedures previously applied to SS washers were applied to PtW squares that had a surface area of 840 mm². In experiments with PtW, the same rates were used for Cuproxat, Green-Shield II, KleenGrow, and ZeroTol 2.0, whereas Clorox was applied at 11.1 and 22.2% product and Virkon S at 1.0 and 2.0% product. The same statistical analyses were applied, but with four and five replications in experiments 1 and 2, respectively.

Based on binomial probabilities, both rates of Clorox and Virkon S had significantly lower recovery of bacterium than the NoSpray and WaterSpray treatments (Fig. 3), but none of these treatments had 0 CFU recovered from all replications (Table 3). Eight of nine replications had 0 CFU recovered from the higher rate of Clorox and both rates of Virkon S, and seven of nine replications had 0 CFU for the lower rate of Clorox. Both rates of Virkon S and the 22.2% Clorox resulted in a large reduction in log CFUs, which makes these viable controls (Table 3).

Based on the ANOVA of treatments with log CFUs greater than zero in three or more replications, treatments were significantly different ($P < 0.0001$) and experiments were not ($P = 0.4653$); as a result, data from both experiments were combined (Table 3). Both rates of KleenGrow and the lower rate of Clorox and Green-Shield II had a reduction in log CFUs of at least 5.5 units lower than the NoSpray and WaterSpray. The ANOVA showed greater delineation in treatment differences than obtained with binomial probabilities. The NoSpray and WaterSpray treatments were not different from each other.

From these results, *P. amygdali* pv. *loropetalii* cells were more difficult to kill on PtW squares than on SS washers. The higher rate of Clorox and both rates of Virkon S provided the most consistent control on PtW. The QACs and the 11.1% Clorox did reduce log CFUs equally. Virkon S is labeled for application on production surfaces in the greenhouse and nursery but has not been used by

TABLE 2
Recovery of *Pseudomonas amygdali* pv. *loropetalii* from inoculated stainless-steel washers sprayed with disinfectant, water, or nothing

Treatment ^x	Product per solution (%)	Experiment	Mean log CFU ^y
NoSpray	0	2	12.35 a
NoSpray	0	1	11.91 a
ZeroTol 2.0	2.0	1	10.82 ab
WaterSpray	100	1	10.12 ab
ZeroTol 2.0	1.0	2	9.85 ab
ZeroTol 2.0	1.0	1	9.60 ab
WaterSpray	100	2	8.87 ab
KleenGrow	1.6	1	8.83 ab
Cuproxat	0.5	2	8.79 ab
Cuproxat	1.0	1	8.65 abc
Cuproxat	0.5	1	7.90 abcd
ZeroTol 2.0	2.0	2	4.19 bcd
Cuproxat	1.0	2	1.80 cd
KleenGrow	1.6	2	1.05 d
KleenGrow	0.8	1	3.57 na ^z
KleenGrow	0.8	2	0 na
Green-Shield II	1.0	1	2.31 na
Green-Shield II	1.0	2	0 na
Green-Shield II	0.5	1	0 na
Green-Shield II	0.5	2	1.66 na
Virkon S	1.0	1	0 na
Virkon S	1.0	2	0 na
Virkon S	0.5	1	1.74 na
Virkon S	0.5	2	0 na
Clorox	11.1	1	0 na
Clorox	11.1	2	0 na
Clorox	0.5	1	0 na
Clorox	0.5	2	0 na

^x Chemical treatments of WaterSpray (reverse-osmosis water), Cuproxat (basic copper sulfate [27.1%]), KleenGrow (didecyl dimethyl ammonium chloride [7.5%]), and ZeroTol 2.0 (hydrogen peroxide [27.1%] and peroxyacetic acid [2.0%]) were spray applied.

^y Analysis of variance (ANOVA) was performed using the MIXED procedure (SAS 9.4). Experiments 1 and 2 were significantly different ($P = 0.0018$) and had three and four replications, respectively. Values with the same letter are not significantly different at $P = 0.05$ based on Dunnett's T3 method for multiple comparison adjustment of least squares means with a standard error of 3.41.

^z na = not analyzed in the ANOVA described previously because the mean and variance were zero in at least one experiment.

plant agriculture industries. Virkon S is used to treat structure surfaces in animal production systems. The 22.2% rate of Clorox is in excess of recommendations given on Environmental Protection Agency (EPA) label registrations of that product.

Limited information is available on efficacy of these disinfectants against bacteria present on wood substrates, making it difficult to project what is a typical response. Knorr (1947) reduced recovery of *Corynebacterium sepedonicum* from contaminated wood crates, which resulted in a reduction of infected seed potatoes using recommended rates of calcium hypochlorite and Roccal, an older second-generation QAC (Knorr 1947). Notably, control of fungal plant pathogens has been variable when applying chlorine and QAC disinfectants to wooden surfaces, frequently providing poor control in many studies but good control in other studies (Copes 2004, 2015; Koponen et al. 1992; Smilanick et al. 2009). A rate of 20% Clorox was needed to achieve control of some fungal pathogens (Copes 2004; Copes and Hendrix 1996). The difference between activity of these products on PtW versus SS surfaces has not been specifically characterized. It is possibly due to reduction in a.i. from the disinfectant reacting with the surface chemistry of substrates and/or due to surface-wetting irregularities whereby bacteria in unwetted cavities are simply not exposed. Other factors such as biofilms, organic residuals, and loss of a.i. due to environmental conditions could also influence efficacy, but these factors were less likely in these in vitro tests.

In these studies, SS surfaces were more easily disinfested of *P. amygdali* pv. *loropetalii* than PtW surfaces. Copes (2004) found that comparable rates of Clorox and QACs disinfested SS and galvanized metal surfaces of *Botrytis cinerea*, whereas higher rates were needed to disinfest PtW (Copes 2004). Although polyethylene plastic products were not tested in this study, these surfaces have been less challenging to treat than PtW (Copes 2004; Koponen et al. 1992). When handling loropetalum stock infected with *P. amygdali* pv. *loropetalii*, it would be preferable to process plant material on metal surfaces, because these surfaces would be more reliably disinfested.

Disinfectant Efficacy on Loropetalum Stems

Of the products that showed activity against *P. amygdali* pv. *loropetalii* on PtW, only KleenGrow lists treatment of vegetatively propagated plant organs on the EPA registered label, and Clorox lists some plant application uses. KleenGrow and Clorox were

tested in separate experiments on loropetalum stem lengths that had a surface area of 1,000 mm². KleenGrow was tested at 0.30, 0.89, 1.48, and 2.08% product and Clorox at 5, 10, 15, 20, and 25% product per solution with WaterSpray as the negative control. The same general methods and statistical procedures described for SS washers were used for sterile loropetalum stems with some variations. All stems were inoculated, treatments applied, and bacteria recovered as an experimental unit of three replications done in succession. A randomized complete block design was used with treatment randomization maintained through each step of the three replications (blocks). Stems were inoculated in the morning hours by submerging a stem in 1% methyl cellulose (Cole Parmer EW-88254-12), setting the stem on hardware cloth to drip excess methyl cellulose into a 100-mm Petri plate, pipetting 250 µl of 0.30 OD₆₀₀ bacterial solution along the stem's length, waiting 5 min to turn the stem over and pipetting 250 µl of bacterial solution along the other side, and waiting another 15 min before adding the lid. Stems were sprayed in the afternoon hours of the same day and incubated overnight at 26°C. Bacteria were recovered the following day. The KleenGrow experiment was repeated once and the Clorox experiment repeated twice.

All four rates of KleenGrow were not significantly different from the WaterSpray or each other ($P = 0.1672$) (Table 4). KleenGrow had activity against *P. amygdali* pv. *loropetalii* in previous tests with SS and PtW surfaces; thus, the same two issues speculated with

Chemical treatment ^x	Product per solution (%)	Mean log CFU ^y
WaterSpray	100	14.43 a
NoSpray	0	13.16 a
ZeroTol 2.0	2	12.18 ab
ZeroTol 2.0	1	12.12 ab
Green-Shield II	1.0	11.33 abc
Cuproxat	0.5	10.86 abc
Cuproxat	1.0	9.26 abcd
KleenGrow	0.8	7.58 bcd
KleenGrow	1.6	7.48 bcd
Green-Shield II	0.5	6.77 cd
Clorox	11.1	5.05 d
Clorox	22.2	0.39 na ^z
Virkon S	1.0	0.51 na
Virkon S	2.0	0.53 na

^x Chemical treatments of WaterSpray (reverse-osmosis water), Cuproxat (basic copper sulfate [27.1%]), Green-Shield II (*n*-alkyl [60% C₁₄, 30% C₁₆, 5% C₁₂, 5% C₁₈] dimethyl ethylbenzyl ammonium chloride [10% active ingredient] and *n*-alkyl [68% C₁₂, 32% C₁₄] dimethyl benzyl ammonium chloride [10% active ingredient]), KleenGrow (didecyl dimethyl ammonium chloride [7.5%]), and ZeroTol 2.0 (hydrogen peroxide [27.1%] and peroxyacetic acid [2.0%]) were spray applied.

^y Analysis of variance (ANOVA) was performed using the MIXED procedure (SAS 9.4). Experiments 1 and 2 were not significantly different ($P = 0.4653$) so were combined for a total of nine replications. Values with the same letter are not significantly different at $P = 0.05$ based on Tukey's multiple comparison adjustment of least squares means with a standard error of 2.64.

^z na = not analyzed in the ANOVA described previously because at least seven of nine replications had 0 CFU and the variance was extremely small.

FIGURE 3

Binomial probability distribution of recovery of *Pseudomonas amygdali* pv. *loropetalii* from pressure-treated wood squares sprayed with a disinfectant, water, or nothing.

PtW, of a load demand and/or incomplete coverage, could be affecting efficacy on loropetalum stems. Irregularities in surface topography resulting in unwetted areas could be more pronounced on stems.

Clorox provided excellent disinfection of stems in two experiments but only moderate disinfection in a third experiment (Table 4). In the two experiments, all but one replication of 10% Clorox had 0 CFU, and all but the 10% Clorox were significantly different than the WaterSpray control (Fig. 4). The increase in the

Treatment ^x	Product per solution (%)	Mean log CFU
WaterSpray	100	11.2060 a ^y
KleenGrow	0.4	9.3136 a
KleenGrow	1.1	9.3182 a
KleenGrow	1.9	8.0202 a
KleenGrow	2.7	9.9355 a
WaterSpray	100	13.5149 c ^z
Clorox	5	7.4251 cd
Clorox	10	3.6143 d
Clorox	15	3.6651 d
Clorox	20	2.1842 d
Clorox	25	4.6643 d

^x Chemical treatments of WaterSpray (reverse-osmosis water) and KleenGrow (didecyl dimethyl ammonium chloride [7.5%]) were spray applied in one experiment and WaterSpray and Clorox (sodium hypochlorite [8.3%]) applied in a second experiment.

^y Analysis of variance (ANOVA) was performed using the MIXED procedure (SAS 9.4). Experiments 1 and 2 were not significantly different ($P = 0.9643$) so were combined for a total of six replications. Values with the same letter are not significantly different at $P = 0.05$ based on Tukey's multiple comparison adjustment of the least squares means with a standard error of 0.8694.

^z ANOVA was performed using the MIXED procedure (SAS 9.4). Results are for experiment 1 only, because results were significantly different in experiments 2 and 3, which are shown in Figure 4. Values with the same letter are not significantly different at $P = 0.05$ based on the least squares means with a standard error of 2.558.

FIGURE 4

Binomial probability distribution of recovery of *Pseudomonas amygdali* pv. *loropetalii* from loropetalum stems sprayed with rates of Clorox or water.

upper probability range to 64.2% for the 10% Clorox treatment reflects the small number of replications used and is likely due to experimental variation. If a greater number of replications had been used, the spread of the probability ranges would shrink and likely result in more pronounced differences between the Clorox treatments and WaterSpray, whereby 10% Clorox could more clearly group with the other Clorox treatments. ANOVA of the third experiment with numerical data found that 10% and higher rates of Clorox were different from WaterSpray (Table 4). Of the three experiments, the one with recoverable log CFUs also had the highest recovery from the WaterSpray treatment, with means (standard deviations) of 13.52 (1.09), 11.80 (1.42), and 8.63 (1.28) log CFUs. Results indicate that 5% Clorox could disinfect stems of *P. amygdali* pv. *loropetalii* as long as inoculum levels are low. Inoculum levels were higher in these studies than would be found naturally on stems.

Clorox should be applied with caution to plant selections, because some plant selections have been injured with daily applications of 5 ppm bleach (Cayanan et al. 2008, 2009). However, Pickens et al. (2019) found loropetalum stem cuttings to be tolerant of 33% bleach with a contact time of 6 min after which the Clorox was rinsed off. A 5% Clorox level would be less likely to cause injury to stem cuttings than a 10% rate. In general, the plant industry would benefit from more research investigating chemical products and application techniques suitable for disinfecting vegetative stem cuttings of plant pathogens without detrimentally injuring plants.

Conclusions and Recommendations

Several disinfectants are commercially available that will disinfect surfaces of *P. amygdali* pv. *loropetalii*, but the choice will depend on the surface being treated. Based on these trials, EPA label recommended rates of Clorox, Green-Shield II, KleenGrow, and Virkon S will disinfect metal surfaces of *P. amygdali* pv. *loropetalii*, whereas only Clorox and Virkon S will comparably disinfect PtW surfaces. Green-Shield II and KleenGrow only caused a moderate reduction on PtW but are an option. ZeroTol 2.0 and Cuproxat caused low mortality of *P. amygdali* pv. *loropetalii* on SS and PtW surfaces. It is recommended that all surfaces be cleaned prior to disinfectant applications to obtain maximum activity.

When handling loropetalum stem cuttings potentially infested with *P. amygdali* pv. *loropetalii*, working surfaces should be routinely disinfected and new damp towels used between batches. Cuttings should be collected in metal containers and processed on metal tabletops or benches when possible because the bacterium is more easily disinfected from metal surfaces. If PtW and polyethylene fabric surfaces are used, select an appropriate product and rate. Clorox appears to be suitable for treating vegetative stem cuttings, but pathogen inoculum levels may affect what rate is needed. Additional research is needed to better characterize efficacy and phytotoxicity, before Clorox can be recommended for treating loropetalum stem cuttings.

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